charged to Deposit Account No. Deposit Account No. 50-1213. If a Petition for extension of time is needed, this paper is to be considered such Petition.

Claims 1, 4, 6, 8-13, 15, 22-35 and 154-216 are presently pending. Claims 2, 3, 5, 7, 14, 16, 17, 154 are cancelled without prejudice or disclaimer, and claims 1, 8 and 22 are amended. Claim 1 is amended to conform to the elected subject matter. Claims 212-216 are added herein. These claims find basis in the instant application and in the parent application. For example basis can be found in the parent application in original claims 1 and 2, page 14, lines 13-16. No new matter has been added.

Prefatory remarks and Further Traverse of the election of species requirement

This application is directed to methods for generating homogeneous compositions of cells that contain a high concentration of one population of immune cells, and also to methods for treatment of diseases by altering immune balance using such compositions. The claims in this application are directed to the methods of producing the such substantially homogenous compositions. As stated at page 12 of the specification:

> Methods of use of regulatory immune cells in autologous cell therapy (ACT) protocols to treat and prevent human disease are provided. The ACT protocols designed to alter the immunoregulatory balance of a patient in order to treat diseases where imbalances in regulatory cells exist. In particular, ACT protocols designed to alter the immunoregulatory balance of a patient in order to treat diseases where imbalances in regulatory cells exist are provided.

There are two major types of regulatory immune cells (formerly under the umbrella of effector cells, but under current terminology **and as defined in the specification)**: Th1 cells, which promote an inflammatory response, and Th2 cells, which act to suppress an inflammatory response. These cells refer to populations of cells in which one cell type predominates; the cell type is defined by its cytokine profile.

The immune system normally maintains these cells in a carefully regulated balance. Imbalances in these regulatory cells are characteristic

of many incurable diseases, such as metastatic cancers, autoimmune, allergic and infectious diseases. For example, cancer patients have an excess of Th2 regulatory cells, which suppress the immune system; whereas autoimmune disease patients have an excess of Th1 regulatory cells, which promote an inflammatory response. The methods herein, provide a means to produce high concentrations of one type of immune cell by collecting lymphoid cells, *differentiating* them into substantially one type of immune cell, and then stimulating them to proliferate to high densities. For treatment, the cells are reinfused into the patient to restore immune balance. The type of cell selected depends upon the disease being treated.

What the Examiner has characterized as "species" are dependent claims directed specific embodiments of the invention. The invention lies in the concept of altering immune balance by administering populations of cells that contain predominantly one type of immune cell in a sufficient concentration to alter immune balance in the recipient using a homogenous population of cells; in methods for production of cells for use in the methods, and in the resulting compositions of cells. Methods for preparing compositions for administration are provided as are methods using the compositions; the compositions are also claimed.

There is no burden to examine and search for the broad concepts, the concept of altering immune function by preparing compositions containing high densities of cells of a particular a type. Furthermore, such claims have already been searched by the Office. The June *et al.* publication (the PCT; see, also U.S. Patent No. 5,858.358, which is of record in this case), which is the closest art of record of which applicant and the Office is aware is directed to methods for stimulating proliferation of cells, but it does not contemplate, teach or suggest the step of **differentiating** the cells into substantially one type of cell, *prior to inducing proliferation*. June *et al.* does not contemplate treating

patients with a composition of substantially homogeneous cells of one type to alter immune balance.

As discussed previously and below, the populations of cells prepared by June *et al.* are not only of relatively low density (1 x 10^6 cells/ml), but are heterogeneous populations (as shown by the data in Table 2 in June *et al.*), which are useless for altering immune balance.

Hence, not only does June *et al.* not anticipate, any of the claims, it does not teach or suggest the instantly claimed methods. June *et al.* does not contemplate methods for altering immune balance nor methods for preparing compositions that contain high concentrations and high densities of populations of cells that contain predominantly one type of immune cell for administration. Hence, there is no art of record that teaches or suggests the generic concepts embodied by the claims. Therefore all of the claims, including dependent claims, which the Examiner characterizes as "species", should be patentable over June *et al.*. If the broad claim is not taught or suggested by the art, dependent claims cannot be taught or suggested.

Traverse of the election of species requirement

First it is noted that as originally filed, a restriction requirement issued dividing the case into five groups. In reliance upon that requirement, four divisional application have been filed. In each of these cases, including this case, further election requirements have been imposed resulting in division of the case into what will (if the requirements are maintained) be 15-20 applications. As discussed above, he claims relate to the same generic concept of producing high concentrations and amounts of homogeneous compositions of cells for infusion in the absence of IL-2 into mammals in order to alter or restore immune system balance. With the exception of original claim 1 and certain claims dependent thereon, the methods involve obtaining immune cells, treating them so that they differentiate into one type of immune cell, and then treating them so that they proliferate into a high number of cells. Dependent claims

specify ways of differentiating them, ways of proliferating them, the products of the differentiation/proliferation methods, and use of the products for treatment. (The only exception to this subject matter are claims that are directed to methods of proliferation for which data predating the June *et al.* publication can be provided.) This subject matter does not warrant 15- 20 patent applications

Turning the specifics of the instant application, in the previous Office Action and election of species was set forth. In response, claims **155-173** directed to methods for generating a regulatory T cells were elected; and if necessary the claims insofar as are directed to methods that produce Th1 cells were elected. It was further noted that claims 1-17, 22-35 and 154-210 read on the elected species.

In the present Office Action, claims 155-173, which were the claims elected, were withdrawn from consideration. Applicant's election cannot be ignored. It is respectfully submitted that the Office cannot withdraw the elected subject matter from consideration.

Furthermore, it is noted that the methods of claims 1-17 and 154-210 insofar as they are directed methods for generating regulatory cells (*i.e.*, Th1, Th2, Th3 cells) encompass the elected species. Each of the independent claims, claims 1, 22, 155, 174 and 197 are directed to methods that include the steps of collecting cells, treating them so that the differentiate into a regulatory subtype, and then expanding the cells.

As discussed in the previous response if this division of claims is maintained, then applicant ultimately could be granted five patents, that include claims with overlapping subject matter. For example, claim 197 is directed to:

> 197. A method for generating a high density of clinically relevant numbers of T lymphoid cells, comprising:
>
> collecting material comprising body fluid or tissue containing mononuclear cells from a mammal;
>
> treating the cells under conditions whereby *ex vivo* differentiation of cells into selected regulatory immune cells is induced; and

contacting, in the absence of exogenous interleukin-2, the material with two or more activating proteins specific for cell surface proteins present on cells in the material and in an amount sufficient to induce *ex vivo* cell expansion, whereby the cells expand in number.

Claim 22 is directed to:

22. (Amended) A method for generating clinically relevant cell numbers of regulatory T lymphoid cells, comprising:

(a) collecting material containing mononuclear T lymphoid cells from a mammal;

(b) activating the cells to alter their cytokine production profile; and

(c) inducing cell proliferation and expanding the cells under conditions that produce at least about 10^{10} cells/liter of a homogeneous population of regulatory T lymphoid cells.

155. A method for generating clinically relevant numbers of regulatory T lymphoid cells for autologous cell therapy, comprising:

(a) collecting material comprising body fluid or tissue containing mononuclear cells from a mammal;

(b) treating the cells to induce differentiation of mononuclear cells into regulatory T cells, wherein regulatory T cells are mononuclear cell that have the ability to control or direct an immune response, but do not act directly as effector cells in the response; and

(c) contacting the resulting differentiated cells with one or more activating proteins specific for cell surface proteins present on the cells in an amount sufficient to induce *ex vivo* cell expansion, whereby clinically relevant numbers of regulatory cells for autologous cell therapy are generated.

Similarly, claim 2 as previously pending (and now claim 1), is directed to a method, comprising:

collecting material comprising body fluid or tissue containing mononuclear cells from a mammal;

treating the cells are under conditions whereby *ex vivo* differentiation of the cells into Th1, Th1-like, Th2-like or Th2 cells is induced; and

contacting, in the absence of exogenous interleukin-2, the material with two or more activating proteins specific for cell surface proteins present on cells

in the material and in an amount sufficient to induce *ex vivo* cell expansion, whereby the cells expand to at least about 10^{10} cells.

Each of these claims could ultimately end up in different patents, since all except claims 22-29 and 31-35 have been withdrawn from consideration as being drawn to non-elected subject matter. Obvious-type double patenting of claims of the later issuing patents cannot be held over the earlier issuing patents. See MPEP 806, paragraph 3, which states:

> [w]here inventions are related as disclosed but are not distinct as claimed, restriction is never proper. Since, if restriction is required by the Office double patenting cannot be held, it is imperative the requirement should never be made where related inventions as claimed are not distinct.

See, also MPEP 804.01, which states:

> 35 U.S.C.121, third sentence, provides that wherein the Office requires restriction, the patent of either the parent or any divisional application thereof conforming to the requirement cannot be used as a reference against the other. This apparent nullification of double patenting as ground of rejection or invalidity in such cases imposes a heavy burden on the Office to guard against erroneous requirements for restriction where the claims define essentially the same inventions in different language and which, if acquiesced in, might result in the issuance of several patents for the same invention.

Hence, if a claims, such as claim 1 issues after claims, such as claims 22 or 197 or 194, obviousness-type double patenting cannot later be held. Applicant could, thus, ultimately obtain at least 4 patents with different expiration dates that claim methods for generating regulatory immune cells (i.e., Th1, Th2, Th1-like, Th2-like etc) by collecting cells, differentiating them into regulatory cells, and then expanding them. The Office will be precluded from requiring terminal disclaimers.

Therefore, examination of claims 1-17, 22-35 and 154-210 insofar as they read on methods for producing regulatory immune cells (Th1, Th2, Th3, Th1-like, Th2-like cells) on the merits is respectfully requested. Moreover, notwithstanding that problems that will arise should the requirement be maintained as presently applied, examination of the elected claims, claims 155-173, is respectfully requested.

THE REJECTION OF CLAIMS 22-29 and 31-35 under 35 U.S.C. §112, FIRST PARAGRAPH

Claims 22-29 and 31-35 under 35 U.S.C. §112, first paragraph, as allegedly containing new matter. The Examiner urges that the specification as originally filed has no basis for "at least about 10^9 cells/liter" in "the context of claim 22." This rejection is respectfully traversed.

As originally filed claim 22 recited:

> 22. A method for generating regulatory immune cells, comprising: collecting material containing mononuclear cells from a mammal; treating the cells to alter their cytokine production profile; and expanding the cells to a clinically relevant number of cells.

As amended in the previous response:

22. (Amended) A method for generating clinically relevant cell numbers of regulatory T lymphoid cells, comprising:

(a) collecting material containing mononuclear T lymphoid cells from a mammal;

(b) activating the cells to alter their cytokine production profile; and

(c) inducing cell proliferation and expanding the cells under conditions that produce high cell density of at least about 10^9 cells/liter and produce clinically relevant number of regulatory T lymphoid cells.

The specification at page 10, lines 1-16, defines a clinically relevant number of cells as:

As used herein, a composition containing a clinically relevant number or population of immune cells is a composition that contains at least 10^9, preferably greater than 10^9, more preferably at least 10^{10} cells, and most preferably more than 10^{10} cells, in which the majority of the cells have a defined regulatory or effector function, such as Th1 cells or Th2 cells or effector cells, such as LAK, TIL and CTL cells. The preferred number of cells will depend upon

the ultimate use for which the composition is intended as will the type of cell. For example, if Th1 cells that are specific for a particular antigen are desired, then the population will contain greater than 50%, preferably greater than 70%, more preferably greater than 80%, most preferably greater than 90-95% of such cells. If the population results from polyclonal expansion, the homogeneous cells will be those that are a particular type or subtype. For uses provided herein, **the cells are preferably in a volume of a liter or less**, more preferably 500 mls or less, even more preferably 250 mls or less and most preferably about 100 mls or less.

The definition is not specific to a particular method, but generally defines the intended meaning of clinically relevant. In response to the Examiner's objection that "clinically relevant" is indefinite, the definition thereof was substituted in place of the words clinically relevant; and the claim slightly modified for grammatical consistency. As presently amended claim 22 recited 10^{10} cells/liter, which similarly finds basis in the specification.

Furthermore, the entire application is directed to methods for generating clinically relevant numbers of immune cells for altering immune system balance by administering such cells. Throughout the application the meaning of this term is made clear by describing the numbers of cells considered clinically relevant and then addressing the issue of a useful volume for administration of the clinically relevant numbers of cells.

Relevant Law

The purpose behind written description requirement is to ensure that the patent applicant had possession of the claimed subject mater at the time of filing of the application In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). The manner in which the specification meets the requirement is not material; it may be met by either an express or an implicit disclosure.

35 U.S.C. §112 requires a written description of the invention. This requirement is distinct from and not coterminous with the enablement requirement:

> The purpose of the 'written description' requirement is broader than to merely explain how to 'make and use'; the applicant must also convey

> with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." Vas-Cath, Inc. v. Mahurkar, 935 F.2d at 1563-64, 19 USPQ2d at 1117 (emphasis in original).

The issue with respect to 35 U.S.C. §112, first paragraph, adequate written description has been stated as:

> [d]oes the specification convey clearly to those skilled in the art, to whom it is addressed, in any way, the information that appellants invented that specific compound [claimed embodiment] Vas-Cath, Inc. v. Mahurkar, at 1115, quoting In re Ruschig, 390 F.2d 1990, at 995-996, 154 USPQ 118 at 123 (CCPA 1967).

A specification must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, *i.e.*, whatever is now claimed. Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991). A written description requirement issue generally involves the question of whether the subject matter of a claim is supported by or conforms to the disclosure of an application as filed. The test for sufficiency of support in a patent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." Ralston Purina Co. v. Far-Mar-Co., Inc., 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed. Cir. 1985) (quoting In re Kaslow, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983)) (see also, MPEP 2163.02).

An objective standard for determining compliance with the written description requirement is "does the description clearly allow persons of skill in the art to recognize that he or she invented what is claimed." In re Gosteli, 872 F.2d 1008, 1012, 10 USPQ.2d 1614, 1618 (Fed. Cir.1989). The Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. In re Wertheim, 541 F.2d 257, 265, 191

USPQ 90, 98 (CCPA 1976); *See also* Ex parte Sorenson, 3 USPQ.2d 1462, 1463 (Bd. Pat.App. & Inter. 1987). By disclosing in a patent application a device that inherently performs a function or has a property, operates according to a theory or has an advantage, a patent application necessarily discloses that function, theory or advantage, even though it says nothing explicit concerning it. The application may later be amended to recite the function, theory or advantage without introducing prohibited new matter. In re Reynolds, 443 F.2d 384, 170 USPQ 94 (CCPA 1971); and In re Smythe, 480 F. 2d 1376, 178 USPQ 279 (CCPA 1973).

Furthermore, the **subject matter of the claim need not be described literally (i.e., using the same terms or inhaec verba)** in order for the disclosure to satisfy the description requirement. If a claim is amended to include subject matter, limitations, or terminology not present in the application as filed, involving a departure from, addition to, or deletion from the disclosure of the application as filed, the examiner should conclude that the claimed subject matter is not described in that application. This conclusion will result in the rejection of the claims affected under 35 U.S.C.112, first paragraph - description requirement, or denial of the benefit of the filing date of a previously filed application, as appropriate.

The guideline promulgated by the U.S. PTO embody these rules:

> In rejecting a claim, set forth express findings of fact regarding the above analysis which support the lack of written description conclusion. These findings should:
>
> (1) identify the claim limitation not described; and
>
> (2) provide reasons why a person skilled in the art at the time the application was filed would not have recognized the description of this limitation in view of the disclosure of the application as filed.

in this instance, there is not basis to conclude that a person skilled in the art at the time the application was filed would not have recognized the description of this limitation in view of the disclosure of the application as filed.

Analysis

The Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. 541 F.2d at 265, 191USPQ at 98. See also Ex parte Sorenson, 3 USPQ2d 1462, 1463 (Bd. Pat. App. & Inter. 1987) (see, also MPEP 2163.04). In this instance, the Examiner has not provided reasons why skilled persons would not recognize disclosure of what is claimed. The skilled artisan would readily glean from the disclosure that a clinically relevant number of cells refers to at least about 10^9 cells in a volume of a liter:

see page 20, lines 1-16, discussed above, which defines the term clinically relevant as referring to a density of at least about 10^9 cells in a liter or less;

see page 21, line 26, - page 22, line 6:

> . . . The therapeutically useful subpopulations are regulatory cells or effector cells and contain clinically relevant numbers of cells, typically at least about 10^9 or more cells, which are preferably in a clinically useful volume (i.e., for infusion) that is one liter or less.
>
> As used herein, a therapeutically effective number or clinically-relevant number ex vivo expanded cells is the number of such cells that is at least sufficient to achieve a desired therapeutic effect, when such cells are used in a particular method of ACT. Typically such number is at least 10^9, and more preferably 10^{10} or more. The precise number will depend upon the cell type and also the intended target or result.

see page 34, lines 18-24, which describes the problems with low densities of cells, and see page 41, lines 1-12:

> T-cells, like most mammalian cells, will grow to a maximum density of 1 x 10^6 cells/ml in tissue culture. Thus, a total of 100 liters of culture

medium would be required to support 100 billion cells. In addition, the 100 liters of medium would have to be replenished regularly to maintain a proper nutrient/waste product balance necessary to keep the cells viable. A method would also be required to keep the 100 liters of medium saturated with oxygen. . . .

1. Administration

The compositions of cell can be administered by any suitable means, including, but not limited to, intravenously, parenterally, or locally. The particular mode selected will depend upon the particular treatment and trafficking of the cells. Intravenous administration is presently preferred. Typically, about 10^{10}-10^{11} cells can be administered in a volume of a 50 ml to 1 liter, preferably about 50 ml to 250 ml., more preferably about 50 ml to 150 ml, and most preferably about 100 ml. The volume will depend upon the disorder treated and the route of administration. The cells may be administered in a single dose or in several doses over selected time intervals in order to titrate the dose, particularly when restoration of immune system balance is the goal.

At page 12, the specification states:

Also provided are methods for producing clinically relevant quantities (i.e., therapeutically effective numbers, typically greater than 10^9, preferably greater than 10^{10}) of autologous specific T cell types for treatment of disease states where a relative deficiency of such cells is observed. In particular, methods for producing clinically relevant numbers of autologous, ex vivo derived Th1 T-cells from patients with disease states where a Th2 cytokine profile predominates such as, but not limited to, infectious and allergic diseases; and autologous, ex vivo derived Th2 T-cells in Th1-dominant diseases, such as, but not limited to ,chronic inflammation and autoimmune diseases, for use in ACT protocols. The resulting cell compositions are provided and the use of the compositions in ACT protocols are provided.

Hence, the skilled artisan would recognize that methods for production of compositions that contain at least 10^9 cells in a liter were contemplated at the time of filing of this application. No new matter has been added.

BASIS IN PARENT APPLICATION AND DEFINITIONS OF EFFECTOR AND REGULATORY CELLS

It is respectfully submitted that pending claims do indeed find basis in the parent application. As discussed above, the test for new matter is that the

specification must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, *i.e.*, whatever is now claimed. Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991). The specification as originally filed conveys with reasonable clarity disclosure that supports all of the pending claims and the claims of this application as originally filed.

In the parent application, U.S. provisional application Serial No. 60/044,693 (converted to a provisional from application Serial No. 08/506,608) claim 1 recites:

1. A method for generating autologous effector immune cells, the method comprising:

collecting material leukocyte containing material from a mammal; and
exposing the leukocyte containing material to mitogenic monoclonal antibodies to induce *in vitro* cell proliferation sufficient for infusion into the mammal for use in an immunotherapy treatment, wherein the *in vitro* cell proliferation is produced without the use of exogenous interleukin-2.

As an aside, because of a restriction requirement claim 1 is now pending one of the co-pending divisional applications. In that case, we will demonstrate actual reduction to practice before the effective filing date of the U.S. application that corresponds to the June *et al.* PCT application at issue in the instant case.

Claim 2 of the parent application recites:

2. The method of claim 1, wherein the leukocyte containing material is caused to differentiate into desired effector cells.

Hence claim 2 corresponds to presently pending claim 1.

Claim 14 recites that the cells are proliferated to "an excess of 1×10^{10} cells."

Claim 7 recites that the cells are Th1-like or Th2-like cells, thereby indicating that as originally filed, what are now called regulatory cells were contemplated to be within the scope of the original claims and to be separately claimed.

The parent application is directed to methods for the production of high concentrations and amounts of homogeneous compositions of immune cells, including Th1, Th2, and also LAK, CTL and TIL cells. In the parent application, the term "effector" cell was used to encompass all types. Dependent claims separated out the Th1 and Th2 cells from the generic type. Claim 1 generically encompassed all types of cells.

In the instant application, the nomenclature, **not** the intended scope of the claims, was modified so that the generic language refers to what was called effector cells in the parent application as "immune cells" Compare claim 1 as originally in the instant case with claim 1 of the parent case. The language "effector" is changed to immune cell. Further, two classes of cells were defined: regulatory immune cells, which are clearly defined (as discussed below) to include Th1 and Th2 cells, which can be identified by their distinct cytokine profiles and which act on other cells; and effector immune cells, which are defined as the LAK and TIL type cells.

The parent specification states at page 7, line 16. that effector cells include Th1, Th2-like cells. The specification describes Th1 and Th2 cells at page 8, lines 27, page 9, line 3, and page 9, lines 20-24; and states at page 9, lines 25-28:

> Accordingly, it is desirable to have the ability to produce large quantities of autologous Th1 T-cells in disease states where a Th2 cytokine profile predominates (infectious disease) and Th2 T-cells in a TH1-dominant disease (chronic inflammation and autoimmune disease).

Methods for differentiation of immune cells into Th1 or Th2 cells are described at page 11, lines 11-19.

The scope of the claims and subject matter of the claims as originally filed and as filed in the instant cased are the same. The definitions were slightly modified in the instant case; but it is clear from the context that the broad claims and dependent claims are the same.

The parent application states, starting at page 6, line 23, that:

> use of ACI protocols will require technology that enables: the generation of homogeneous populations of immune effector cells [*i.e.*, cells that include TILs, LAKs, CTLs, Th1, Th2 cells]; the consistent growth of effector cells to clinically relevant dosages (*i.e.*, greater than 10^{10} cells) without the use of IL-2; . . . and the ability to reinfuse the cells without the need for systemic infusion of IL-2. Furthermore additional *in vitro* differentiation strategies are need to broaden the types of cells available for ACI protocols.

The present invention addresses each of these requirements, disclosing a method to differentiate Th1 or TH2 cells *in vitro* and grow these cells to clinically relevant numbers without the use of IL-2.

This provides clear unequivocal basis for claim 1 in the present application.

The instant application has been rewritten for clarity, not to add new matter to the claims, and to provide additional supporting examples. To distinguish between differentiation of cells to produce LAKs, TILs and CTLs, from differentiation to produce Th1, Th2 and Th3 cells and subcategories thereof, different nomenclature has been adopted. The same cells are encompassed by the claims; their names are different. Changing the names of claimed subject matter does not add new matter if the substance remains substantially the same.

Similarly, claim 155 and 197 find basis in the parent application. Claim 155 recites:

> A method for generating clinically relevant numbers of regulatory T lymphoid cells for autologous cell therapy, comprising:
> (a) collecting material comprising body fluid or tissue containing mononuclear cells from a mammal;
> (b) treating the cells to induce differentiation of mononuclear cells into regulatory T cells, wherein regulatory T cells are mononuclear cell that have the ability to control or direct an immune response, but do not act directly as effector cells in the response; and
> (c) contacting the resulting differentiated cells with one or more activating proteins specific for cell surface proteins present on the cells in an amount sufficient to induce *ex vivo* cell expansion, whereby clinically relevant numbers of regulatory cells for autologous cell therapy are generated.

Basis for claim 155 may be found in original claim 2, which includes the steps of obtaining the cells (see, also page 10, lines 15-20, which describes the step of obtaining mononuclear cells), causing them to differentiate (see, also page 10, line 25, - page 11, line 21 which describes means to cause differentiation of the collected cells into various cell types, including using Il-2) and expanding the differentiated cells. Basis for claim 197 may be found in original claim 2.

Claims 26-35 also find basis in the parent application. As discussed above, the application is directed to methods for producing homogenous populations of cell by collecting mononuclear cells, causing them to differentiate by altering their cytokine profiles to produce regulatory cells, and then expanding them to high numbers. Claims 26-30 specifically recite that the differentiated cells are Th1 or Th2 or Th1-like or Th2-like. Hence basis for claims 26-30 may be found in original claims 2, 7-14, and in the specification at page 7, lines 9-15, page 8, line 25, - page 10, line 7, and page 10, line 25, - page 11, line 19.

Therefore, the scope and content of claims of the instant application finds basis in the application as originally filed. Furthermore, as discussed below, the instant application clearly defines the terms sufficiently to meet the requirement of 35 U.S.C. §112, second paragraph.

REJECTION OF CLAIMS 1, 2, 17, 22, 23, 27 and 30 UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 1, 2, 17, and 22-35 are rejected as being indefinite under 35 U.S.C. § 112, second paragraph. Various bases for this rejection are set forth and each is discussed in turn. Reconsideration of the grounds for rejection is respectfully requested in view of the amendments of the claims and the following remarks.

1. Claim 22

Claim 22 is rejected as being indefinite in the recitation of "regulatory immune cell" because the definition in the specification is allegedly unclear. The specification defines regulatory immune cells as follows:

> As used herein, a regulatory immune cell is any mononuclear cell with a defined cytokine production profile and in which such cytokine profile does not directly mediate an effector function. A regulatory immune cell is a mononuclear cell that has the ability to control or direct an immune response, but does not act as an effector cell in the response. Regulatory immune cells exert their regulatory function by virtue of the cytokines they produce and can be classified by virtue of their cytokine production profile. For example, regulatory immune cells that produce IL-2 and IFN-γ, but do not produce IL-4 are termed "Th1" cells. Regulatory immune cells that produce IL-4 and IL-10, but do not produce IFN-γ are termed "Th2" cells. Regulatory immune cells that produce TGF-β, IL-10 and IFN-γ, but do not produce IL-2 or IL-4 are termed "Th3" cells. Cells that produce Th1, Th2 and Th3 cytokine profiles occur in CD4+ and CD8+ cell populations. Cells that produce IL-2, IL-4 and IFN-γ are thought to be precursors of Th1 and Th2 cells and are designated "Th0" cells. Populations of cells that produce a majority of Th1 cytokines are designated "Th1-like"; populations producing a majority of the Th2 cytokines are designated Th2-like"; those producing a majority of Th3 cytokines are designated "Th3-like". Thus, each composition, although containing a heterogeneous population of cells, will have the properties that are substantially similar, with respect to cytokine, to the particular Th subset.
>
> It is understood that this list of T- cells is exemplary only, and any other definable population, array or subtype of T cells that can be expanded by the methods herein to clinically relevant numbers are intended herein.

An effector cell is defined as:

> As used herein, effector cells are mononuclear cells that have the ability to directly eliminate pathogens or tumor cells. Such cells include, but are not limited to, LAK cells, MAK cells and other mononuclear phagocytes, TILs, CTLs and antibody-producing B cells and other such cells.

Hence from the above definition is it clear that Th1, Th2 and Th3 cells, as well as populations of cells designated Th1-like, Th2-like and Th-3-like are regulatory immune cells. These cells all have distinct cytokine profiles and are

defined by their cytokine profiles and their function is by virtue of these cytokine profiles. In contrast, effector cells are mononuclear cells that are not regulatory cells and that like LAK, TILS, MAKs, CTLs and B-cells directly eliminate pathogens by virtue of their direct interaction with pathogens and tumor cells.

Relevant Law

When "one skilled in the art would understand all of the language in the claims when read in light of the specification," a claim is not indefinite. Rosemount Inc. v. Beckman Instruments, Inc., 727 F.2d 1540, 1547, 221 USPQ 1, 7 (Fed. Cir. 1984), Caterpillar Tractor Co. v. Berco, S.P.A., 714 F.2d 1110, 1116, 219 USPQ 185, 188 (Fed. Cir. 1983). The claims are definite if they "make clear what subject matter they encompass and thus what the patent precludes others from doing". In re Spiller, 182 USPQ 614 (CCPA 1974). The requirements of § 112, second paragraph are met when one can "examine the claims to see whether the invention's metes and bounds can be adequately determined from the claim languages". In re Goffe, 188 USPQ 131 (CCPA 1975).

Analysis

First, as discussed above, the specification defines regulatory immune cells as follows:

> a regulatory immune cell is any mononuclear cell with a defined cytokine production profile and in which such cytokine profile does not directly mediate an effector function. A regulatory immune cell is a mononuclear cell that has the ability to control or direct an immune response, but does not act as an effector cell in the response. Regulatory immune cells exert their regulatory function *by virtue of the cytokines they produce and can be classified by virtue of their cytokine production profile*. For example, regulatory immune cells that produce IL-2 and IFN-γ, but do not produce IL-4 are termed "Th1" cells. Regulatory immune cells that produce IL-4 and IL-10, but do not produce IFN-γ are termed "Th2" cells. Regulatory immune cells that produce TGF-β, IL-10 and IFN-γ, but do not produce IL-2 or IL-4 are termed "Th3" cells. Cells that produce Th1, Th2 and Th3 cytokine profiles occur in CD4+ and CD8+ cell populations . . ..

Furthermore, these cells are contrasted in the specification to "effector cells, such as LAK, TIL and CTL cells" (see, e.g., page 20), which the specification defines (page 20, lines 23-26) as follows:

> As used herein, effector cells are mononuclear cells that have the ability to directly eliminate pathogens or tumor cells. Such cells include, but are not limited to, LAK cells, MAK cells and other mononuclear phagocytes, TILs, CTLs and antibody-producing B cells and other such cells.

Responses are mediated by virtue of their interaction with a pathogen or tumor cell, not by virtue of their secretion of a cytokine.

The specification continues at page 24, line 27 *et seq.* as follows:

> Regulatory immune cells control the nature of an immune response to pathogens [see, Mosmann, *et al.* (1986) J. Immunol. 136:2348; Cherwinski, *et al.* (1987) J. Exp. Med. 166:1229; and Del Prete, *et al.* (1991) J. Clin. Invest. 88:346]. The different types of responses are attributable to the heterogeneity of $CD4^+$ T cells. $CD4^+$ cells can be subdivided according to their cytokine expression profiles. These cells are derived from a common precursor, Th0, which can produce Th1, Th2 and Th3 cytokines [see, Firestein, *et al.* (1989) J. Immunol. 143:518]. As noted above, Th1 clones produce IL-2, INF-γ, lymphotoxin and other factors responsible for promoting delayed-type hypersensitivity reactions characteristic of cell-mediated immunity. These cells do not express IL-4 or IL-5. Th1 cells promote cell-mediated inflammatory reactions, support macrophage activation, immunoglobulin (Ig) isotype switching to IgG2a and activate cytotoxic function.
>
> Th2 clones produce cytokines, such as IL-4, Il-5, IL-6, IL-10 and IL-13, and thus direct humoral immune responses, and also promote allergic type responses. Th2 cells do not express IL-2 and IFN-γ. Th2 cells provide help for B-cell activation, for switching to the IgG1 and IgE isotypes and for antibody production [see, e.g., Mosmann *et al.* (1989) Annu. Rev. Immunol. 7:145]. Th3 cell produce IL-4, IL-10 and TGF-β.
>
> The cytokines produced by Th1 and Th2 cells are mutually inhibitory. Th1 cytokines inhibit the proliferation of Th2 cells and Th2 cytokines inhibit Th1 cytokine synthesis [see, e.g., Fiorentino, *et al.* (1989) Med. 170:2081 (1989). This cross regulation results in a polarized Th1 or Th2 immune response to pathogens that can result in host resistance or susceptibility to infection . . ..

Thus, the nature of regulatory immune cells as opposed to effector cells, which are cells that **directly eliminate pathogens or tumor cells** is clear from the specification.

The Examiner notes that Th1 cells secrete lymphotoxin and urges that this contradicts the definition. It is respectfully submitted that this is in line with the definition provided above. The Th1 cells are mediating activity by virtue of secretion of the lymphotoxin, **not** by direct interaction with cells.

Also as noted previously, Liblau *et al.* does not teach that Th1 or Th2 cells are "effector cells" as alleged by the Examiner. Liblau *et al.* refers to Th1 and Th2 cells as having "**effector function**", this is distinct from the definition of effector and regulatory cells as defined in the instant application. Since these cells participate in the effector phase of an immune response they have effector function, but they do not directly cause the elimination of a target antigen, such as a true effector cell, such as a CTL or TIL cell.

Furthermore, there is no teaching in Liblau *et al.* that pathogenicity in IDDM is caused by Th1 cells directly destroying islet cells such as is known to occur by immune effector cells. Rather, Liblau teaches that **Th1 cells mediate** (i.e. have a regulatory function) the pathogenesis of IDDM **by the types of cytokines they produce**. The ability of Th1 or Th2 cells to mediate disease is characterized by Liblau *et al.* as "effector function" (see page 34, first column, second paragraph, line 8; page 37, legend to Figure 1, line 12), but it does not meant that the cells are effector cells. They are acting in accord with the definition of regulatory cells in the instant application.

There is a distinction in the art between "effector function" as used by Liblau *et al.* in which the cells participate in the effector phase of an immune response, and effector cells, such as, CTLs, B cells or phagocytic monocytes, which directly act to eliminate the target antigen. **Regulatory immune cells participat indirectly in immun r sp ns s by elaborati n of cytokin s.** Although Liblau *et al.* describes that "effector function" is associated with

effector and regulatory immune cells, this does not contradict nor confuse the definitions given in the instant application. The definitions in the instant application render it very clear that cells that mediate their function by virtue of their cytokine profile are regulatory cells; and cells the mediate their function by direct interaction with a target are effector cells.

Consistent with Liblau *et al.*, the specification provides separate definitions for regulatory and effector immune cells at page 19 as:

> any mononuclear cell with a defined cytokine production profile and in which such cytokine profile **does not directly mediate an effector function**. A regulatory immune cell is a mononuclear cell that has the ability to control or direct an immune response, but does not act as an effector cell in the response. Regulatory immune cells exert their regulatory function by virtue of the cytokines they produce and can be classified by virtue of their cytokine production profile.

The above definition requires a regulatory immune cell to have a defined cytokine profile, where the cytokines do not directly mediate an effector function. The contemplation of a regulatory cell that indirectly mediates an effector function as defined in the specification is consistent with Liblau *et al.* which demonstrates "effector function" for regulatory T cells. The definition of a regulatory immune cell in the specification also expressly distinguishes it from an effector immune cell as defined on page 19 as:

> mononuclear cells that have the ability to directly eliminate pathogens or tumor cells. Such cells include, but are not limited to, LAK cells, MAK cells and other mononuclear phagocytes, TILs, CTLs and antibody-producing B cells and other such cells.

Thus, the specification, clearly distinguishes an immune regulatory cell from an immune effector cell. The definition in the specification of a regulatory immune cell encompasses Th1, Th2 and Th3 cells, and this is not inconsistent with the concept of "effector function" as described by Liblau *et al.*

2. **Claim 22** is allegedly indefinite in the recitation of "clinically relevant." As discussed previously and above, this term is clearly defined in the specification; no where in the specification is the definition limited to

compositions. The term is defined per se and clearly intended to encompass the products of the methods. The definition in the specification is set forth above. Furthermore, the meaning of this term is clearly set forth throughout the specification. For example, at page 11, lines 9-11 recite:

> Methods for generating the compositions containing the clinically relevant numbers of immune cells for use in adoptive immunotherapy are provided.

At page 12, lines 5-16, recite:

> Also provided are methods for producing clinically relevant quantities (i.e., therapeutically effective numbers, **typically greater than 10^9, preferably greater than 10^{10}**) of autologous specific T cell types for treatment of disease states where a relative deficiency of such cells is observed. In particular, methods for producing clinically relevant numbers of autologous, ex vivo derived Th1 T-cells from patients with disease states where a Th2 cytokine profile predominates such as, but not limited to, infectious and allergic diseases; and autologous, ex vivo derived Th2 T-cells in Th1-dominant diseases, such as, but not limited to ,chronic inflammation and autoimmune diseases, for use in ACT protocols. The resulting cell compositions are provided and the use of the compositions in ACT protocols are provided.

At page 13, the specification recites:

> The methods involve collecting peripheral blood mononuclear cells from a patient and then expanding the cells by appropriate activation and then mitogenic stimulation with a cell surface specific proteins or proteins under conditions whereby clinically relevant numbers of the expanded cell type are produced [typically 10^9, preferably 10^{10}, more preferably 10^{11}, or more depending upon the cell type and ultimate application]. If the collected cells are not differentiated in vivo or require further differentiation, then following collection and prior to expansion, the method includes activating and causing differentiation of the cells ex vivo under conditions whereby at least some of the cells differentiate into regulatory or effector cells or other cell types.

Then as noted above, the definition provided in the specification states that:

> a clinically relevant number or population of immune cells is a composition that contains at least 10^9, preferably greater than 10^9, more preferably at least 10^{10} cells, and most preferably more than 10^{10} cells, in which the majority of the cells have a defined regulatory or effector function, such as Th1 cells or Th2 cells or effector cells, such as LAK, TIL and CTL cells.

Hence, in accord with the requisites of 35 U.S.C. §112, second paragraph, it is clear that a clinically relevant number of cells is at least 10^9 cells. It is further clear that in preferred embodiments, these cells are contained

> in preferably in a volume of a liter or less, more preferably 500 mls or less, even more preferably 250 mls or less and most preferably about 100 mls or less.

REJECTION OF CLAIMS 22-28, 31, 32, 34 and 35 UNDER 35 U.S.C. § 102(b)

Claims 22-28, 31, 32, 34 and 35 are rejected under 35 U.S.C. § 102(b) as being anticipated by June *et al.* (WO 94/29436) because June *et al.* allegedly teaches:

> the method of claim 22 wherein unfractionated T cells or CD4+ or CD8+ cells are expanded to clinically relevant numbers by treatment with antiCD3 antibody followed by antiCD28 or antiCTLA4 antibody (see abstract, pages 4-9, 13, claims 1-28, 30, 37).

The Examiner urges that Figures 1 and 2 show about expansion of the cells to greater than 10^{10} and that the cells were treated with IL-2, which the Examiner urges leads to the development of a Th1 phenotype (the Examiner points to Table 2 to support this conclusion). The Examiner further urges that June *et al.* teaches that cells in Table 2 were treated with IL-2 and the resulting cells exhibit a Th1 profile because they produce IFNγ. The Examiner also urges that prior to treatment the cells can be treated with antigen to induce *ex vivo* differentiation of said cells into antigen specific effector cells (see page 9, first complete paragraph) and that the cells can be purified (see page 29 and 30).

The rejection is respectfully traversed.

First, it is noted that June *et al.* is not a reference under 35 U.S.C. §102(b) against the claims in this application, and particularly against claims 22 and claims dependent thereon. While not conceding that claims 22-25 do not find basis, at the very least claims 26-35 find exact basis in the parent application. As to those claims at least, as well as the other pending claims in this case (claims 1, 155, 174 and 197 and claims dependent thereon), June *et al.* is not a reference under 35 U.S.C. §102(b).

The arguments in the previous response are herein incorporated in their entirety. Clarification of certain points is set forth below.

The instant claims and application are directed to methods of producing clinically relevant numbers of cells and methods for producing populations of cells that are sufficiently stable and homogeneous to be useful for clinical application. Claim 22 recites a method that includes the steps of:

(a) collecting material containing mononuclear T lymphoid cells from a mammal;

(b) activating the T lymphoid cells to alter their cytokine production profile by causing differentiation of the cells; and

(c) inducing cell proliferation and expanding the cells under conditions that produce at least about 10^{10} cells/liter of a homogeneous population of regulatory T lymphoid cells.

Relevant Law

Rejection for anticipation requires, as a first step in the inquiry, that all the elements of the claimed invention be described in a single reference [see, e.g., In re Spada, 15 USPQ2d 1655 (Fed. Cir, 1990), In re Bond, 15 USPQ 1566 (Fed. Cir. 1990), Soundscriber Corp. v. U.S.. 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, Richardson v. Suzuki Motor Co., 868 F.2d 1226,1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989)].

Disclosure of June *et al.*

June *et al.* discloses a method for inducing a population of T cells to proliferate by providing a first signal to activate the cells and then a second

signal to stimulate proliferation. Proliferation is sustained by periodic reactivation and restimulation. Cell densities substantially greater than 10^6 are not disclosed. Further, June *et al.* does not disclose or teach a method in which cells are differentiated into regulatory cells prior to expansion. **Any steps in which cells are treated with IL-2 are performed AFTER the expansion step not prior to this step.**

June *et al.* alleges that its method can be used to expand T cells in long term tissue culture to obtain a population increased in number **not in concentration** from about 100 to about 100,000 fold over the original starting cell population. Figures 1-3 purport to show that the method actually results in cell numbers could exceed 1 x 10^{10} cells, but do not teach or suggest preparation of such cells at a density of about 10^9/liter or 10^{10} liters or more.

Also, June *et al.* does not disclose growth of a clinically relevant number of T cells. The results in Figures 1-3 are merely extrapolations of potential growth from a single flask. For example, page 27, line 8-9 of June *et al.* only mentions a single flask and the cells are "resuspended every day and diluted to 0.5 x 10^6 cells/ml." If the cells are stored at each resuspension, the resulting 10^{10} cells will be in a at density of about 0.5 x 10^6 cells/ml.

June *et al.* does not disclose growth of T cells to high cell density of 10^{10} cells/Liter to obtain clinically relevant numbers of cells. The reference teaches growing the cells at a cell density maintained at 0.5 x 10^6/ml" (page 28, line 31). Thus, June *et al.* does teach every element of the instant claims, and, therefore, does not anticipate any of claims . Each of these claims require production of clinically relevant number of cells and expansion of the cells to produce cell densities of at least about 1 x 10^{10} cells/Liter.

June *et al.* does not disclose expansion of T cells to high cell density. In addition, June *et al.* does not anticipate these claims because it does not disclose preparation of or expansion of regulatory immune cells to produce homogeneous populations as defined and claims in the instant application. In

Table 2. June *et al.* discloses expansion of CD4+ cells using anti-CD3 + anti-CD28 or IL-2 and evaluates the cytokines produced during culture (see Table 2, page 32).

Stimulation of CD4+ cells with the combination of antibodies used by June *et al.* does not generally produce regulatory cells, and certainly does nor result in a substantially homogeneous population. June admits that the expanded CD4+ cells have unstable cytokine profile (see page 31, lines 26-29).

As defined in the specification at page 19, lines 4-19:

> . . . regulatory immune cells that produce IL-2 and IFN-γ, but do not produce IL-4 are termed "Th1" cells. Regulatory immune cells that produce IL-4 and IL-10, but do not produce IFN-γ are termed "Th2" cells. Regulatory immune cells that produce TGF-β, IL-10 and IFN-γ, but do not produce IL-2 or IL-4 are termed "Th3" cells. Cells that produce Th1, Th2 and Th3 cytokine profiles occur in CD4+ and CD8+ cell populations. Cells that produce IL-2, IL-4 and IFN-γ are thought to be precursors of Th1 and Th2 cells and are designated "Th0" cells.

Thus, the T cells activated by June *et al.*,are characterized by an unstable profile of cytokines and do not meet the definition of a regulatory T lymphoid cell as required by the claims. As discussed previously, the cells of June *et al.* are probably CD4+ cells of a precursor Th0 phenotype because, as shown in the Table, the cells produce IL-2, IL-4 and IFN-γ, which production is characteristic of Th0 cells, which are precursor cells not regulatory immune cells.

Thus, June *et al.* does not anticipate any of the claims because it does not disclose expansion of T cells to clinically relevant numbers under conditions that produce high cell density. Furthermore, June *et al.* does not anticipate these claims or any of the pending claims because it does not disclose or suggest the step of differentiation into regulatory cells prior to the activation/proliferation step by contacting the cells with two or more proteins.

Comments by the Examiner

Turning the comments by the Examiner, page 9 first complete paragraph, does not teach differentiation of T cells into regulatory immune cells prior to

expansion. In this paragraph, June *et al.* describes antigen specific activation of a population of cells. Preparing antigen-specific cells does not involve differentiation of cells to produce regulatory immune cells as claimed in the instant claims.

With respect to Table 2, June *et al.* specifically discloses use of antibodies to CD3 or CD2 to and antibodies to CD28 to stimulate cell proliferation. Alternatively, June *et al.* uses IL-2 with anti-CD28 for induction of proliferation. Table 2 presents a comparison of the results of stimulation of proliferation using antibodies and either IL-2 or anti-CD28 as the co-stimulus **to induce activation/proliferation, not to induce differentation.** There is no separate step of inducing differentiation and then addition of activation/proliferation signals. June *et al.* uses activation/proliferation signals, such as anti-CD3 or IL-2 and anti-CD28. Hence, there is not differentiation step as urged by the Examiner.

IL-2 and anti-CD28 are added co-stimulants; IL-2 is not added for achieving differentiation, but to promote activation/proliferation. This is not inherently the same as adding Il-2 for differentiation followed by two or more signals that promote activation/proliferation. The difference can be seen in Table 2. The cytokine profiles of the resulting expanded cells are shown in Table 2 over several cycles of stimulation. The data in the Table clearly demonstrate that the combination of adding Il-2 or anti-CD3 **WITH** anti-CD28, followed by several additional cycles of anti-CD28 is not the same method as claimed in the instant application. It does not result in regulatory cells as defined in the instant application, and certainly not in a homogeneous population of cells. The step of adding IL-2 with anti-CD28 is **not a step that causes differentiation into regulatory cells**. The resulting cells even at that stage produce Il-4 as well as IFN-γ and IL-2, and the amount of Il-4 increases with each cycle. Furthermore, the additional cycles in which only anti-CD28 are added do not meet the limitations of the instant claims, which require contacting

with two or more activating proteins specific for cell surface proteins. If the first activation cycle is somehow construed to be a differentiation cycle, the subsequent cycles do not meet the limitations of the instant claims which require two ore more activating proteins (to ensure a proliferative response).

It can be seen that by the third cycle of stimulation in the June *et al.* experiment, the cells are producing IL-2, IFN-γ **AND** IL-4. Hence the resulting population of cells does not have characteristics of a homogeneous population of regulatory cells, but rather has a mixed phenotype, which as discussed above is characteristic of undifferentiated precursor T_0 cells. These cells, which produce IL-4 are clearly not Th1 cells. Since they produce INF-γ, they cannot be Th2 cells. Hence there is no evidence that the June *et al.* produces Th1 cells, and certainly does not produce a homogeneous population of Th1 cells. Therefore, not only does June *et al.* not explicitly contemplate a method of differentiating cells followed by proliferation, June *et al.* does not inherently perform the instantly claimed methods.

As discussed herein and in the previous response, June *et al.* does not teach methods for producing homogenous populations of expanded cells. June *et al.* is directed to methods for expanding lymphocytes in the absence of IL-2. Nowhere in June *et al.* is there a suggestion to <u>first</u> differentiate the cells, and then expand the differentiated cells.

Therefore because June *et al.* does not disclose preparation of cells at a density of 10^{10} cells/liter or greater nor differentiation of cells to a regulatory phenotype prior to the expansion, nor treatment to induce differentiation followed by treatment with two or more activating proteins to induce proliferation, June *et al.* cannot and does not anticipate any of the pending claims.

REJECTION OF CLAIMS 22-28, 31, 32, 34 and 35 UNDER 35 U.S.C. § 102(e)

Claims 22-28, 31, 32, 34 and 35 are rejected under 35 U.S.C. § 102(e) as being anticipated by June *et al.* (U.S. Patent No. 5,858,358) for the same reasons and on the same basis as set forth for the June *et al.* PCT application. U.S. Patent No. 5,858,358 claims priority to U.S. application Serial No. 08/073,223, which is the priority application to which International PCT application No. 94/29436 claims priority. Both the PCT and application upon which the issued patent is based were filed on 3 June 1994. While not having compared the disclosures of the PCT application and the U.S. patent word-for-word, they appear to be substantially identical in disclosure.

Hence for the reasons set forth above and in previous responses with respect to June *et al.*, International PCT application No. 94/29436, this rejection is respectfully traversed.

THE REJECTION OF CLAIMS 22-28, 31, 32, 34 and 35 UNDER 35 U.S.C. § 103(a)

Claims 22-28, 31, 32, 34 and 35 are rejected under 35 U.S.C. § 103 as being unpatentable over June *et al.*, in view of Cracauer *et al.* (US patent No. 4,804,628). It is alleged that June *et al.* teaches the method of claim 22 and Cracauer *et al.* allegedly teaches hollow fiber bioreactors and the use of such devices for efficiently growing large numbers of cells in vitro. It is concluded that one of ordinary skill in the art would have been motivated to do the aforementioned because Cracauer *et al.* teach that "hollow fiber culture devices have been proven to be ideal for the maintenance of many types of cells at high densities in culture."

The rejection is respectfully traversed.

The Claims

Claims 22-28, 31, 32, 34 and 35 are discussed above. Claim 35 is directed to the method of claim 22 where expansion is conducted in a hollow fiber bioreactor.

June *et al.*

June *et al.* teaches a method for inducing a population of T cells to proliferate by providing a first signal to activate the cells and then a second signal to stimulate proliferation. The reference teaches use of antibodies to CD3 or CD2 (or in a control experiment IL-2) to activate the cells and antibodies to CD28 to stimulate cell proliferation. June *et al.* also teaches purifying populations of CD4+ or CD8+ T cells and growing the cells.

June *et al.* does not teach the step of expanding the cells to therapeutically useful or clinically relevant numbers, nor does June *et al.* teach or suggest a method in which cells are differentiated prior to the activation and proliferation step . June *et al.* states that T cells can be grown in long term tissue culture to obtain a population increased in number from about 100 to about 100,000 fold over the original starting cell population, but does not suggest preparation of clinically relevant numbers or therapeutically effective concentrations. June *et al.* also states that the method could be used to grow large numbers of T cells (e.g., more that 10^{10} cells), but does not teach or suggest means for achieving high cell densities.

The data shown in Figures 1-3 of June *et al.* are merely extrapolations of potential growth from a single flask. At page 27, line 8-9 of June *et al.* there is mention of a flask where the cells are "resuspended every day and diluted to 0.5×10^6 cells/ml." Thus, June *et al.* does not teach or suggest growth of T cells under conditions that produce high cell density to obtain clinically relevant numbers of cells.

June *et al.* also teaches the use of the T cell proliferation method for obtaining a population of CD4+ or $CD8^+$ T cells and, in the case of CD4+ cells, June *et al.* evaluates the cytokines produced by the cells at different stages of growth. This teaching, however, says nothing about the production of regulatory T lymphoid cells and does not teach or suggest adding a step of differentiation before the activation and proliferation step.

Although the Examiner alleges that June *et al.* teaches expansion of regulatory immune cells, it is respectfully submitted that June *et al.* does not teach, suggest or contemplate differentiation of cells into regulatory cells prior to such expansion. As discussed above, there is no reference to "regulatory" cells at page 6 of June *et al.* as alleged or anywhere else in the reference nor does page 9 suggest differentiation into regulatory cells prior to expansion.

Stimulation of CD4+ cells with the combination of antibodies used by June *et al.* does not generally produce regulatory cells and June *et al.* admits that the expanded CD4+ cells have an unstable cytokine profile (see page 31, lines 26-29).

Cracauer *et al.*

Cracauer *et al.* teaches a hollow fiber cell culture device that includes a hollow fiber cartridge having a shell and a plurality of capillaries extending through the shell with at least some capillaries having semi-permeable walls. A cell culturing space is located between the shell and the capillaries. The device includes a chamber containing a second medium supply fluidly connected to the cell culturing space.

Cracauer *et al.* does not teach or suggest use of its hollow fiber cell culture for growing clinically relevant numbers of immune cells or T lymphoid cells, or for that matter any type of lymphoid cell. The device of Cracauer *et al.* is not taught to be suitable for growing lymphoid cells at densities exceeding 1 x 10^8 cells/ml.

The Examiner has failed to set forth a case of *prima facie* obviousness

(1) Relevant law

In order to set forth a prima facie case of obviousness under 35 U.S.C. § 103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (ACS Hospital Systems, Inc. v. Montefiore Hospital, 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must

actually teach or suggest the claimed invention. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. Ex parte Gerlach, 212 USPQ 471 (BPAI 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art" In re Keller, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination (ACS Hosp. Systems, Inc. v Montefiore Hosp. 732 F.2d 1572, 1577. 221 USPQ 329, 933 (Fed. Cir. 1984)).

(2) There would not have been motivation to have combined the teachings of June *et al.* with those of Cracauer *et al.*

June *et al.* does not contemplate preparation of high densities of cells, and thus, provides no motivation to have selected the device of Cracauer *et al.* Cracauer *et al.* provides no suggestion for growing T lymphoid cells in its device. Therefore, the ordinarily skilled artisan would not have had any motivation to have added an additional step to the method of June *et al.* and have expanded the cells in the device of Cracauer *et al.*

(3) The combination of references does not result in the claimed subject matter

June *et al.* teaches a method of growing T lymphocytes, CD4+ and CD8+ T cells at relatively low cell densities of about 1×10^5 cells/ml- 0.5×10^6 cells/ml using a combination of mitogenic antibodies in the absence of IL-2. June *et al.*, however, does not teach or suggest a method for obtaining clinically relevant numbers of T lymphoid cells nor expansion of such cells under conditions that produce high cell density, an element recited in all of the claims. As discussed above, June *et al.* fails to teach or suggest a differentiation step (required in all pending claims) prior to expansion. June *et al.* does not teach or suggest growth to produce high cell density that exceed 1×10^9 cells/Liter.

June *et al.* also does not teach or suggest a method of producing regulatory T cells or a method whereby the regulatory T cells are expanded under conditions that produce high cell density to result in clinically relevant numbers of cells. At most, June *et al.* teaches proliferation of CD4+ cells using anti-CD3 + anti-CD28 and evaluation of the cytokines produced. The resulting cells do not have a stable cytokine profile (see page 31, lines 26-29) nor a profile resembling a homogeneous population of regulatory cells nor any regulatory cells. As discussed above, the cytokine profiles of the expanded cells do not meet the definition of a homogeneous population of regulatory immune cells, but rather appear to result in heterogenous populatoins of cells.

Cracauer *et al.* does not cure the deficiencies in the teachings of June *et al.* because Cracauer *et al.* merely teaches a hollow fiber device. There is no teaching or suggestion in Cracauer *et al.* to use the device for expanding T cells at high cell density nor how to adapt the device to achieve such a goal. Further, Cracauer *et al.* does not teach or suggest a method for producing regulatory cells nor the step of effecting differentiation prior to expansion.

Therefore, Cracauer does not cure the deficiencies in the teachings of June *et al.*, and the combination of references does not teach or suggest the instantly claimed methods.

The Rejection over June *et al.* in view of Cracauer *et al.* is Based on Improper Use of Hindsight.

The disclosure of the applicant cannot be used to hunt through the prior art for the claimed elements and then combine them as claimed. In re Laskowski, 871 F.2d 115, 117, 10 USPQ2d 1397, 1398 (Fed. Cir. 1989). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" W.L.

Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

It appears that the Examiner has combined the teachings of the prior art with those of the instant application. Because the cited prior art fails to teach or suggest any of these essential requirements or provide any motivation to have combined the references, nor the results of such combination, for the rejection to set forth a prima facie case of obviousness, it necessarily must have utilized the teachings of the specification to make the combination.

Neither June *et al.* nor Cracauer *et al.*, singly or in combination, teaches or suggests growth of T lymphoid cells to clinically relevant numbers under conditions that produce high cell density; and neither suggests a step of differentiating cells to produce regulatory cells prior to activation.

* * *

In view of the above remarks and the amendments and remarks of record, consideration and allowance of the application are respectfully requested.

Respectfully submitted,
HELLER EHRMAN WHITE & MCAULIFFE LLP

By: _____
Stephanie Seidman
Registration No. 33,779

Attorney Docket No. 24731-500B
Address all correspondence to:
HELLER EHRMAN WHITE & MCAULIFFE LLP
4250 Executive Square, 7th Floor
La Jolla, CA 92037
Telephone: 858 450-8400
Facsimile: 858 587-5360
EMAIL:sseidman@HEWM.com